Windows to the Deep Cruise Summary
Introduction
Carolyn Ruppel
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At great water depths (more than about 2000 meters), the chosen study area on the U.S. Atlantic margin is underlain by a vast reserve of gas trapped in ice-like hydrate deposits and by free gas that can migrate freely through the sediments. Where faults, salt diapirs, and sedimentary structures perturb the gas hydrate reservoir, there is significant potential for the occurrence of conventional chemosynthetic seep communities or non-conventional seeps that might be marked at the seafloor by more subtle clues about the circulation of fluids and gas through the underlying sediments.

The Windows to the Deep expedition focused on exploration of three areas: (a) The Blake Ridge Diapir; (b) the Cape Fear Diapir and surrounding region; and (c) the Blake Ridge in the vicinity of a large sediment wave field. The criteria for the choice of these sites were the presence of gas or gas hydrate in the shallow subsurface, evidence for missing gas or active seafloor emission of methane, and/or the presence of features that could be expected to channel the flow of fluids and gas from deep in the sedimentary section.

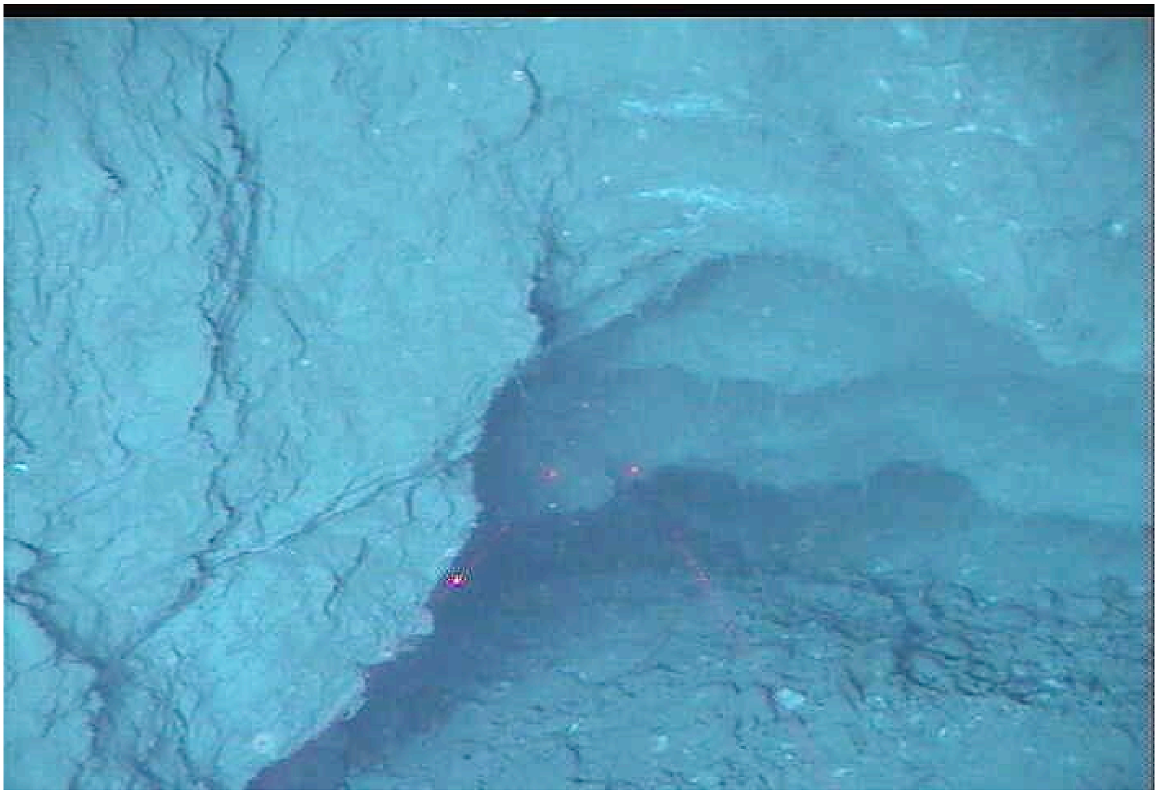
The red dots show the locations of the dives completed on The Windows to the Deep expedition. Green symbols indicate drilling sites for ODP Leg 164 in 1995.

Alvin Dives

The expedition featured seven Alvin dives carried out at water depths of 2150 meters to nearly 3500 meters on and near all of the target areas. While no new methane seeps were discovered, three dives on the Blake Ridge Diapir gave researchers the opportunity to expand their exploration of this known methane seep and to map the distribution of live clams, dead clams, and mussel fields in greater detail. During the dives, scientists collected quantitative samples of clam populations for studies linking clam distribution and health to the geochemistry of underlying sediments, acquired new mussel samples for histological research, and used push cores to sample sediments and bacterial mats for geochemical analyses and DNA- and RNA-based microbial community studies.


Push coring patches of live clams on the Blake Ridge diapir.

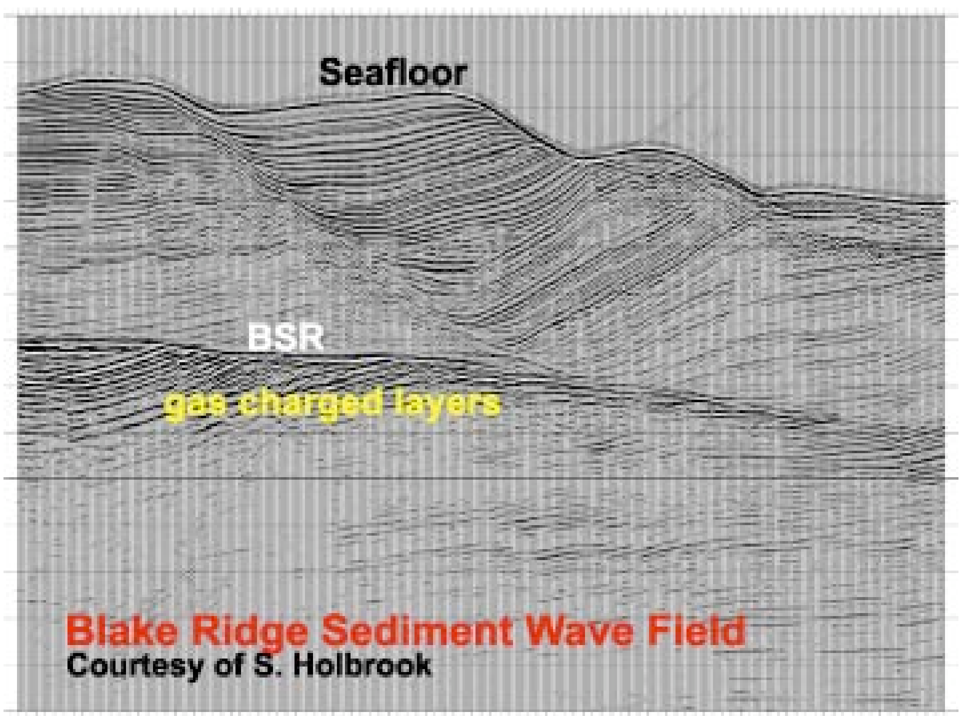
The dives on the Blake Ridge Diapir also permitted researchers to revisit gas hydrate that was first observed on the seafloor in 2001. The gas hydrate area had been disturbed and partially obscured by sediment in the past 22 months, and the gas hydrate deposit itself may also be smaller, although clear pictures could not be obtained. During the 2001 cruise, researchers were unable to sample the ice shrimp that congregated on and around the gas hydrate. This time Alvin acquired mature individuals of both the large and small varieties of shrimp at this location. Methane seeped from the seafloor during acquisition of one push core near the gas hydrate location, and the frenzied shrimp seemed to seek either the source of fresh methane or small creatures stirred up by the coring as soon as the push cores were extracted.



The rocks shown in the foreground in this photo obscure hydrate hidden under the ledge and out of range of the camera. The hydrate deposit appears to be much smaller in 2003, or at least far more hidden, than in 2001. Compare this image with the one on the mission summary page for The Windows to the Deep expedition.

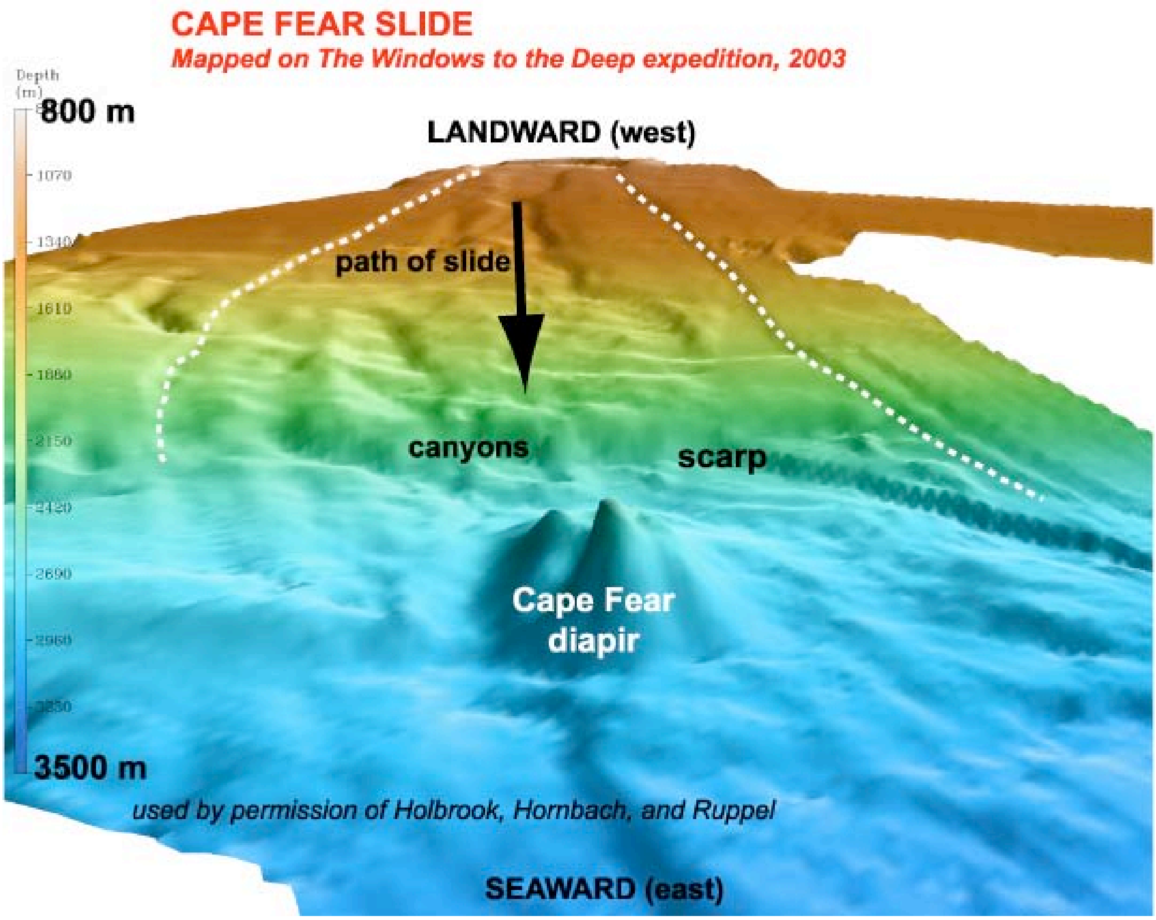
A single “ice shrimp” in the vicinity of the hydrate on the Blake Ridge diapir.

Four dives were conducted on promising features in the Cape Fear area (Dives 3911, 3913, 3914) and on the northern slope of the Blake Ridge (Dive 3908). The latter site was chosen because seismic images collected by Holbrook in 2000 revealed the presence of methane chimneys and missing gas in the subsurface in the vicinity of a large sediment wave field on the Blake Ridge. The northern side of the ridge, which is the erosional flank, was considered the best target for features associated with the escape of deep gas. In addition, Chief Scientist Ruppel and collaborating scientist Holbrook have for several years been involved in a community effort to propose future deep-sea drilling of this area to study the dynamics of the Blake Ridge gas hydrate reservoir. Having found no signs of seafloor seepage at this location during the Alvin dive, researchers infer that focused venting may not occur, that the seep sites may be difficult to find during limited time in manned submersibles, or that gas venting may have been an older event that no longer manifests itself at the seafloor. Possibly a better strategy for exploring such areas is use of a ROV that can survey systematically around the clock.



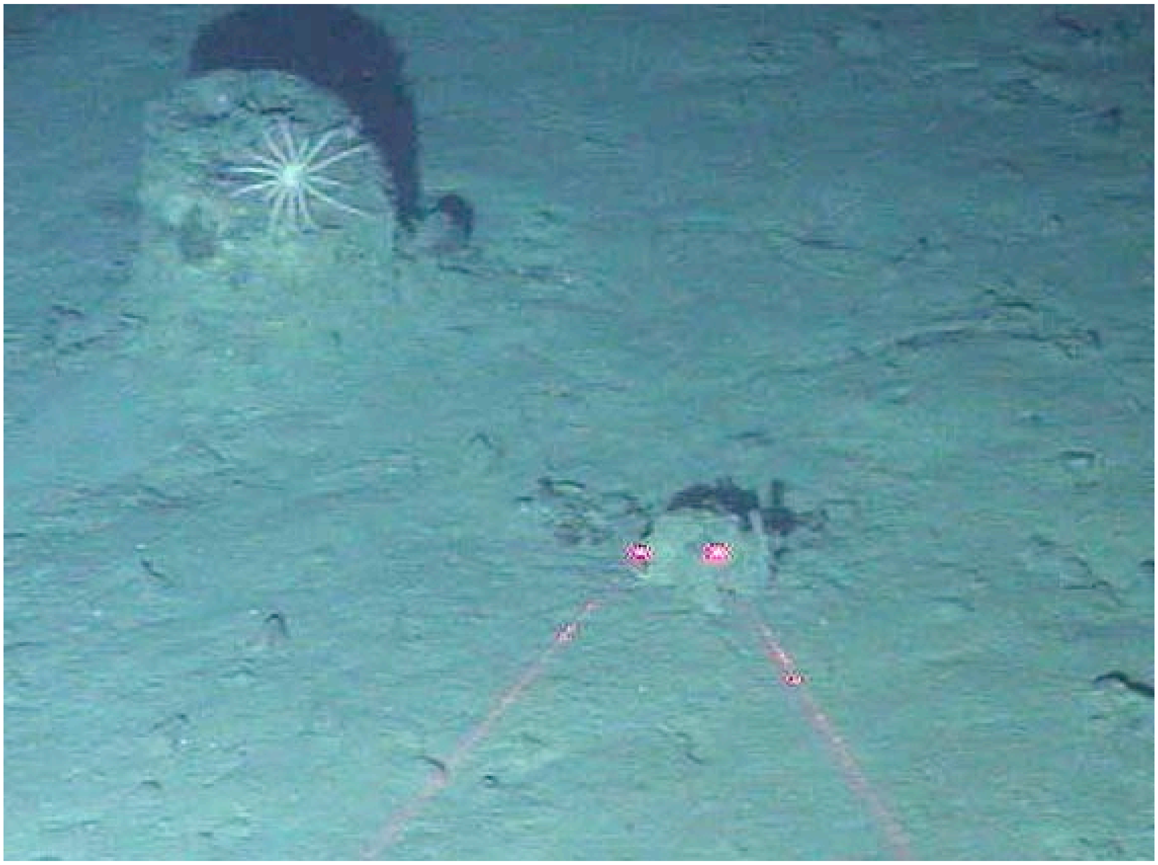
High-resolution seismic image of part of the Blake Ridge Depression sediment wave field, showing a strong BSR (a bottom simulating reflector that marks the base of the free gas zone) on the left, weakening to the right. This image was collected during site survey work conducted by Steve Holbrook in 2000 with support from the National Science Foundation and the Department of Energy.

In the Cape Fear area, researchers explored the top and flank of the Cape Fear Diapir and a fault scarp that appeared to cap shallow gas within the Cape Fear slide area. From the standpoint of methane venting, the most promising dive explored the top of the diapir. Alvin’s occupants found octopods (which consume shellfish, the dominant organisms in chemosynthetic communities on the Blake Ridge diapir) and a small amount of broken shell near their landing site, but an hour-long search revealed no seep site. Another find on top of the diapir was a field of sedimented debris, including one feature that may be a sediment-covered authigenic carbonate containing shell detritus. Such carbonate only forms in the presence of seep-related microbial activity. While this interpretation is highly speculative, several clues imply that the top of the Cape Fear Diapir may now or may have in the past hosted seep sites.



New Seabeam map of the Cape Fear slide and the breached diapir, here shown in a three-dimensional image compiled by Matt Hornbach. The image is from the perspective of an observer looking due west.

The other dives in the Cape Fear area explored a canyon close to where free gas was predicted to intersect the seafloor and a rubble field within the Cape Fear slide, the largest submarine slide on the U.S. Atlantic coast. Conventional seep communities were not encountered on either dive, but the ascent up the side of the Cape Fear Diapir did provide direct access to a fault zone probably associated with the emplacement of the diapir. During this dive, researchers also discovered meter-high mudstone and mud chimneys that were pervasively burrowed in all directions and that contained organisms that ducked back into the chimney when the Alvin manipulator arm reached out to sample.



Mud chimneys of unknown origin were found during a dive on the flank of the Cape Fear Diapir.

GEOPHYSICAL EXPLORATION

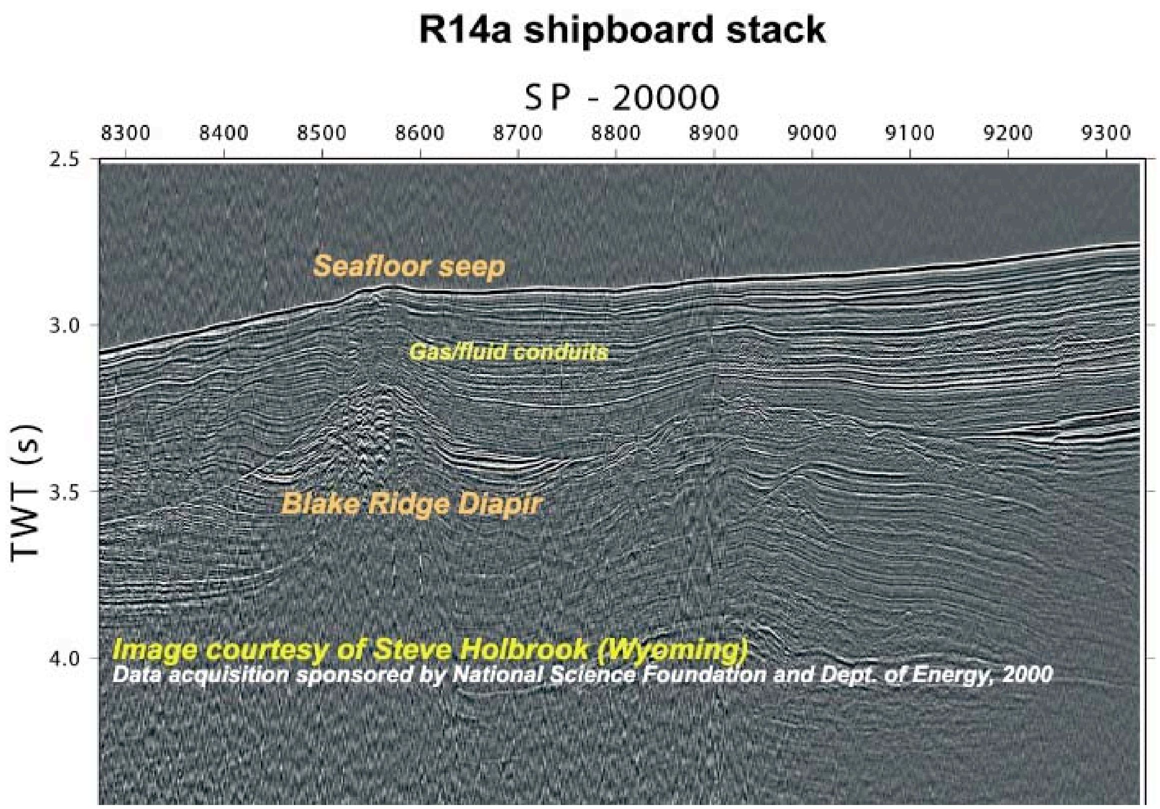
Carolyn Ruppel
Georgia Institute of Technology

Windows to the Deep had an active nighttime program of Seabeam bathymetric mapping and 3.5 kHz subbottom profiling owing largely to the efforts of University of Wyoming graduate student Matt Hornbach and his advisor, Windows to the Deep collaborator Steve Holbrook. These surveys and some of the Alvin dives produced new results that enhanced our understanding of methane seeps, the relationship of salt diapirism to the region's geology and gas hydrate dynamics, and structural features associated with the Cape Fear landslide.

This map shows the total area mapped by Seabeam during The Windows to the Deep expedition. Note the closely spaced tracks over the feature at the far southerly end, which is the Blake Ridge diapir.

Methane seeps

The Blake Ridge Diapir was the focus of intensive seismic studies as early as the 1992, and even older, lower resolution data clearly imaged methane chimneys in the shallow sediments overlying the salt core of the diapir. In 1995, the diapir was drilled by the Ocean Drilling Program during ODP Leg 164, in which both Ruppel and Holbrook were participants. The drilling results clearly revealed the top of the diapir as an area of methane venting active enough to sustain a chemosynthetic community. High resolution seismic data obtained in 2000 by Steve Holbrook as part of a NSF- and DOE-funded project further constrained the location and morphology of fluid conduits beneath the diapir. The new 3.5 kHz subbottom images acquired during Windows to the Deep provide a critical link between seafloor observations at the top of the flow system and the high resolution data that best define structures in the deeper (greater than several tens of meters below the seafloor) part of the section. Using techniques borrowed from traditional seismic surveys, we conducted 3.5 kHz surveys with shiptracks spaced about 40 meters apart to obtain a three-dimensional image of the diapir. Processing of these data may permit researchers to track individual faults and gas seeps that sustain different parts of the seafloor chemosynthetic system.



Raw high-resolution seismic data collected over the Blake Ridge Diapir by Steve Holbrook in 2000 reveal the location of focused methane chimneys beneath the seafloor chemosynthetic seep community.

The fact that methane seeps were not more widely discovered in promising areas during The Windows to the Deep expedition has also provided new lessons for the researchers. While the original criteria for narrowing the choice of seep sites remain valid, it is clear that a particular combination of geologic features is probably needed to set the stage for the rapid fluid flow and persistent gas flux needed to sustain chemosynthetic communities.

During the last dive on the Blake Ridge Diapir, Alvin recovered an authigenic carbonate sample. While this material litters the seafloor in some parts of the site, there had not been a previous opportunity to obtain carbon isotopic analyses that will constrain the source of the carbonate. It is expected that this sample, which is being analyzed by graduate student Bill Gilhooly and Dr. Stephen Macko of the University of Virginia, will yield carbon isotopic values consistent with a microbial source for the carbonate.

Authigenic carbonate pieces collected during The Windows to the Deep expedition. Photo by Amy Eisin.

Salt diapirs

The association of methane seeps with salt diapirs can be explained by the impact of salt's high thermal conductivity on thermal gradients in the sediments. The presence of salt beneath sediment causes the gas hydrate zone to thin and free gas to lie closer to the seafloor. During The Windows to the Deep Expedition, we conducted the most complete survey ever undertaken of the Cape Fear Diapir, a dramatic bathymetric feature that disrupts the bottom simulating reflector (which marks the base of the free gas zone). A dive on the flank of the Cape Fear diapir discovered a rock face marked by slickensides, which are striations associated with fault movement. During the same dive, the observers found strange mud chimneys emerging from the seafloor. Morphologically, the seafloor had several other inexplicable features, including hummocky bathymetry with numerous small basins and isolated pits containing discolored sediment and a green flocculant material. The possible connection between these seafloor observations and earlier USGS imaging surveys that refer to a dissolution pitted surface for the Cape Fear Diapir merits further study.

Chief Scientist Dr. Carolyn Ruppel and Joint Oceanographic Institutions intern Anna Henderson discuss rock samples obtained during dives on and near the Cape Fear Diapir. The striations on the large rock in the foreground indicate that the rock likely originated in a fault zone. Note the gas hydrate-themed ODP Leg 164 T-shirt (methane + water make gas hydrate) being worn by Ruppel.


Researchers discovered strange pits with green-tinged sediments on the Cape Fear diapir. Here the Alvin manipulator arm takes a push core sample from one of the pits.

Cape Fear Submarine Slide

The Cape Fear slide, the largest submarine slide on the U.S. East Coast, occurred approximately 20,000 years ago and may have developed in response to seafloor destabilization related to methane hydrates and associated free gas. The timing of the slide also roughly coincides with the end of the last major glaciation. The Windows to the Deep Expedition compiled the most accurate and complete map ever made of the entire Cape Fear slide. The new map reveals the location of the major bounding scarps, the upper headwall scarp and subsidiary scarps, and more minor slides within the larger slide. The amount of gas trapped beneath the Cape Fear slide is large compared to that beneath other similar slides, and the new map will lay the groundwork for a major future study of this region and the hydrate-related processes associated with submarine slope failure.

GEOCHEMISTRY

Bill Gilhooly
University of Virginia

For the geochemistry program, thirty-five push cores were collected during five Alvin dives. Extracting water from the sediment cores was one of the most time-consuming tasks and yielded 250 water samples (link to 'working around the clock' http://www.oceanexplorer.noaa.gov/explorations/03windows/logs/jul27/jul27.html). The major sampling strategy was to collect deep-sea sediments in the proximity of and below healthy clam beds. These clams are host to chemosynthetic bacteria that utilize hydrogen sulfide generated *in situ* by sulfate reducing bacteria. Intensive sulfur cycling in these anoxic sediments (sediments with little or no oxygen) underlying oxygenated bottom waters is fueled by bacterial consumption of marine organic matter in the sediments and/or any of a number of light hydrocarbons such as the methane gas observed to vent from the seafloor and accumulate as gas hydrate.

Shipboard analysis of the pore waters included measurement of redox potential (Eh) and salinity. Redox potential indicates the interface between oxygenated sediment (oxic) and sediment with very little or no oxygen (anoxic). The cores typically below the first 2 cm changed from a light to dark color, indicating anaerobic (occurring in the absence of free oxygen) conditions. The Blake Ridge is situated above a system of salt diapirs, which may supply highly saline waters to the upper sediments. Thus, we also measured pore water salinity of the surface sediments, anticipating higher salinity. Multiple analyses of the pore waters indicated a salinity of approximately 34 parts per thousand, which is that of standard seawater. Additional land-based laboratory analyses will be conducted to help describe the microbial activity that supports the chemosynthetic symbionts in the bivalve gills, and to assist in the characterization of the environment where clams were present.

BIOLOGY

Clam Ecology at Cold Seeps

Taylor Heyl, Anne Mills, Cindy Lee Van Dover
College of William and Mary

One of the main objectives for the biologists on this cruise was sampling of deep-sea clams from the Blake Ridge Diapir to determine how environmental parameters correlate with the density and condition of these organisms. During this cruise, 48 push cores of sediment from four different habitat types – live clams, dead clams, a combination of live and dead clams and a background site with no clams – were sampled using *DSV Alvin* during three dives on the diapir. In live clam patches, as many as 45 20-mm-long clams occupied a surface area with a diameter of 60 mm.

Redox profiles were measured as the cores were brought on deck to locate the oxic-anoxic (the interface between oxygenated sediment and sediment with very little or no oxygen) interface below the sediment surface, and the researchers found a positive correlation between the depth of the oxic-anoxic zone in the sediment and clam density. Sediment intervals (2-cm) from these push cores will be analyzed for chemical parameters (pore water sulfide, sulfate, particulate organic carbon, and methane measurements in collaboration with chemists) and physical parameters (grain size). Biological parameters (reproductive condition and parasite burdens in the clams) will be determined primarily from histological analyses in the laboratory. Reproductive condition of clams and the presence or absence of parasites are both indicators of the health of a population. A total of 623 clams were preserved for condition indices or histology.

Redox measurements being conducted on recovered push core by Bill Gilhooly of University of Virginia and Taylor Heyl of William and Mary. Photo by Margaret Olsen.

Parasite Burdens in Blake Ridge Mussels

Megan Ward and CL Van Dover
The College of William & Mary

The *Bathymodiolus heckerae* mussels collected in 2001 from the Blake Ridge Diapir were infected with a pathogenic viral inclusion in the gut that causes deterioration of the epithelial tissues. Regions of mass mortality in the mussel population at Blake Ridge are evident from dive observations, but the cause of the mortality is unknown. During the Windows to the Deep expedition, we collected fresh samples of *B. heckerae* to determine if the disease had progressed in the live population. We had marked the locations of healthy and diseased mussel beds in 2001 and returned to these same sites for the new samples. The healthy area was characterized by little sediment cover, few empty mussel shells, and many juveniles; the unhealthy region had more sediment cover, fewer juveniles, and many empty mussel shells. Samples were fixed for later analysis using light microscopy and transmission electron microscopy (TEM). To analyze the progression of the viral infection, gut tissue will be examined histologically to determine viral infection prevalence (% individuals infected) and infection density (number of viral-inclusions per 500 μm^2 per individual). These measures will be compared to values determined for 2001 samples. To identify the specific type of virus in the Blake Ridge mussels, tissues will also be examined at high magnification using TEM.

Live mussels (left) and dead mussels (right) shown during the 2003 visits to the Blake Ridge chemosynthetic community. One focus of the biological work is the impact of a virus first discovered in the mussels as an outcome of the 2001 cruise.

Sampling mussels with the Alvin manipulator arm during the 2001 Deep East expedition that conducted the initial exploration of the chemosynthetic communities on the Blake Ridge diapir.

MICROBIOLOGY

Microbial Ecology

Heath Mills and Rob Martinez
Georgia Institute of Technology

Taylor Heyl
The College of William and Mary

Diving to new habitats always brings with it the appeal of discovery. After seven *Alvin* dives that collected push cores from a variety of habitats, there is potential for promising new discoveries about seafloor microbial communities. Unlike the observers aboard *Alvin*, microbiologists rarely get to see their new discoveries through the small portholes. They instead rely on molecular techniques performed in land-based laboratories to fully understand the microbial impact on an environment. However, simply observing the habitats where the cores were taken has provided a certain level of excitement about the prospects of unlocking secret identities of the unseen organisms of the Blake Ridge ecosystem.

The Windows to the Deep expedition obtained sediment push cores from clam and mussel beds, white bacterial mats, and in close proximity to an exposed hydrate mound. Due to a lack of knowledge of the microbial populations at Blake Ridge, these cores will certainly provide several new lineages unique only to the Blake Ridge. At the very least, the microbes identified will provide a glimpse of different biological processes active at different locations on the sea bottom. Will we be able to identify the differences in the microbial communities living at live clam beds versus dead clam beds? Are there different populations at the mussel bed? Will the microbial communities at the relatively recently exposed hydrate mound be similar to the communities identified in the more permanent and older hydrate mounds in the Gulf of Mexico and Cascadia Margin? These questions will begin to be answered after many months of hard work and the collaborative efforts of other scientists.

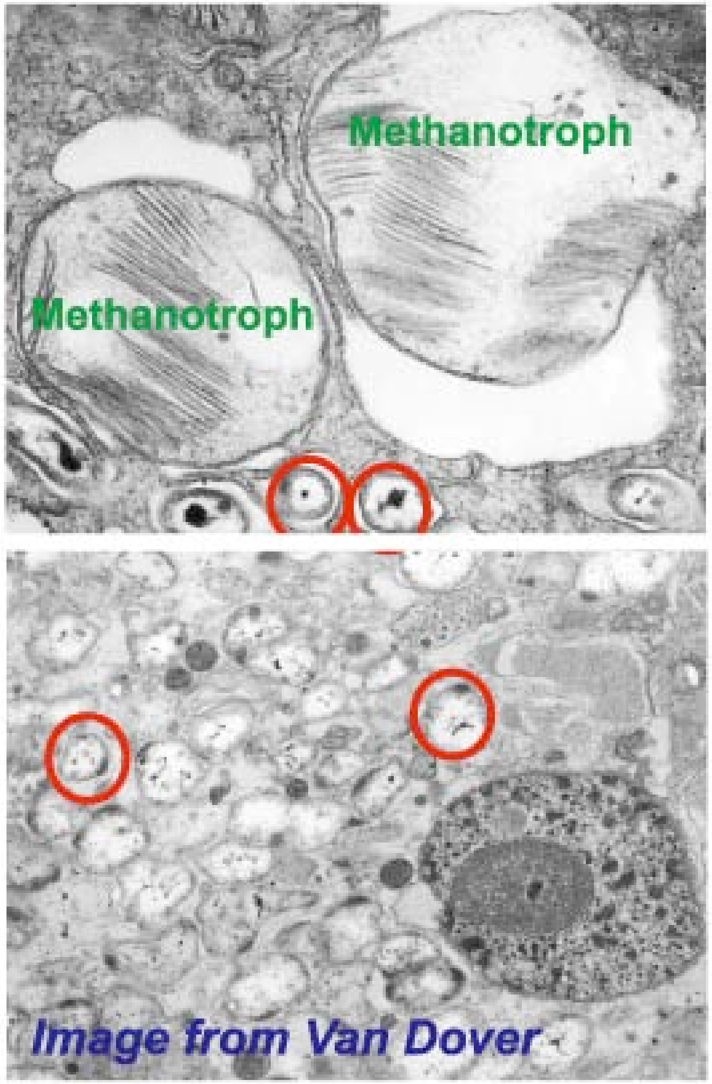
Push core sampling of a thick, white bacterial mat associated with live clams. The red laser dots are **10** cm apart.

Sulfide-Oxidizing Bacteria

Rachel Horak, C L Van Dover, and Mark Forsyth
The College of William and Mary

In 1999, Bernhard and Bowser reported that a foraminiferan (single celled protests with shells – see http://www.ucmp.berkeley.edu/foram/foramintro.html for more information) species (*Nonionella stella*) abundant in *Beggiatoa* sp. bacterial mats of the Santa Barbara Basin at 600 m, sequestered chloroplasts (plant cell inclusion bodies that contain chlorophyll, a photosynthetic pigment). That same year, Tapley et al. (1999) suggested that the spontaneous inorganic oxidation of sulfide in seawater produces weak, visible light through chemiluminescence, light produced without heat. This paper suggested that light is produced when sulfide-rich fluids from hydrothermal vents or seeps come into contact with oxidized seawater and are oxidized to sulfate. Chemosynthetic bacteria also oxidize sulfide to sulfate, and in the metabolic process, generate energy. We are testing the hypothesis that sulfide-oxidizing bacteria can produce light that may be biologically useful. In the case of *Beggiatoa* sp. in the Santa Barbara Basin, we hypothesize that this light could be used by chloroplasts in the foraminifera.

During The Windows to the Deep cruise, we collected a variety of sulfide-oxidizing bacteria species that will be used in further lab studies, including DNA analysis for bioluminescence genes. In addition, live *Vesicomya* cf. *venusta* clams and *Bathymodiolus heckerae* mussels, each of which contain sulfide-oxidizing bacteria within their gill tissue, were collected. The symbiotic sulfide-oxidizing bacteria oxidize sulfide to sulfate and in the process generate useable energy for the clam or mussel. The gill tissue from the mussels and clams was frozen for DNA analysis for the presence of the *lux* operon, the mechanism bacteria use to produce bioluminescence. Samples of bacterial mats found at the Blake Ridge Diapir were acquired to augment the studies of sulfide-oxidizing bacteria. While studies of the bacterial population diversity will be completed at Georgia Tech, part of the sample will be used for DNA analysis of the sulfide-oxidizing species, looking for the presence of the *lux* operon in the same fashion as in the symbiotic bacteria. Samples of the bacterial mat were also kept alive to be grown in pure culture for bioluminescence tests.



These images show symbiotic bacteria found in the gills of mussels (top) and clams (bottom). The red circles show thiotrophic (sulfide-oxidizing) bacteria, which are present in both the clams and the mussels. The mussels contain both methanotrophic bacteria (bacteria that require methane for survival) and thiotrophic bacteria.